ARCHIWUM OCHRONY ŚRODOWISKA
1 109–120 1995

PL ISSN 0324-8461

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EXTRAPOLATION OF THE EFFECTS OF GLYPHOSATE FROM THE LABORATORY TO THE FIELD

INTRODUCTION

Hazards caused by the release of chemicals into the terrestrial environment can be assessed by the use of laboratory data on toxicity to different species. For the assessment of safe concentrations the chronic NOECs may be used to extrapolate from the population level of species to the field by the use of statistically based extrapolation methods which estimate hazardous or safe concentrations. Alternatively, it is possible to elaborate hazardous or safe concentrations by the use of multispecies tests. This work is part of a project in which impact factors for single pesticides are developed. The final goal is to set up environmental indicators for the side-effects of all crop-pesticide combinations at a national scale. The study was initiated by the Danish Environmental Protection Agency [11].

Several extrapolation methods based on species sensitivity distribution have been developed [12, 15]. In this study the Wagner and Løkke [23] method was applied on the herbicide glyphosate. The aim was to assess a "safe" concentration for all species in the agroecosystem and its surroundings which may be affected by spray drift. The exposure/ecotoxicity ratio is calculated as PEC/PNEC, where PEC is the predicted environmental concentration, and PNEC is the predicted no effect concentration or dose estimated by the extrapolation method.

Glyphosate is chosen as a model test compound because it has selective action on plants and low toxicity to other groups of organism. This toxicity pattern allows for the investigation of two key questions on the use of distribution-based extrapolation methods: Do single species toxicity test results fit log-normal distributions, and do species of largely different taxonomy belong to the same log-normal distribution?

METHOD

The toxicity data were retrieved from the open literature, and from sources in the Danish Environmental Protection Agency [3]. Papers which presented sublethal toxicity data (NOECs) based on concentration-effect relationships were selected. The data are listed in Table 1 which shows the species, the NOEC values and their units, the exposure routes, the toxicity endpoints, the exposure time, and the literature references.

The data were tested for normality by using the RANK and the UNIVARIATE procedures [19]. If normal-distributed, the data appear as a straight line if plotted against the normal scores of their ranks. The UNIVARIATE procedure computes the Shapiro-Wilk statistic, W. By using the log-normal extrapolation formula given by Wagner and Løkke [23], the lower statistic tolerance limit K_p is determined so that with the probability 95% no more than 5% of all species in the terrestrial system have a NOEC value lower than K_p,

$$K_p = \exp(\bar{x}_m - s_m \cdot k), \tag{1}$$

where $\bar{x}_m$ is the mean of the natural logarithm of the toxicity values, s_m is the standard deviation, and k a factor dependent on the number of species, N, and the degree of confidence. The method requires at least five test species to be used to obtain reliable results.

The PEC/PNEC ratio is determined by the expression:

$$\text{PEC/PNEC} = \text{RFD}/(f_{\text{exp}} \cdot \text{PNEC}), \tag{2}$$

where RFD is the recommended field dosage, and f_{exp} is a factor which describes some exposure components. In the present study, $f_{\text{exp}} \cdot \text{PNEC} = K_p$. NOEC toxicity data on mammals and birds were converted to the area based unit D (kg/ha) by the expression:

$$D = \frac{c_{\text{lit}} \cdot M \cdot p \cdot 100}{m \cdot f_p \cdot f_d}, \tag{3}$$

where c_{lit} is the NOEC value cited in the literature, M is the body weight of the test animal, m is the daily intake of food, p is the plant biomass per area unit, f_p is the fraction in percent of pesticides which hits the plants (85%), and f_d is an exposure factor taking into account the degradation, sorption etc. ($f_d = 0.1$ for glyphosate).

In cases where the NOEC is based on the concentration of the chemical in the food, the equation (4) is used

$$D = \frac{c_{\text{lit}} \cdot p \cdot 100}{f_p \cdot f_d}. \tag{4}$$

For soil dwelling organisms the NOEC values are converted to an area based unit (kg/ha) by assuming that the herbicide is distributed in the upper soil layer, $h = 1$ cm. NOECs derived from experiments in soil were transformed by the equation:

$$D = \frac{c_{\mathrm{lit}} \cdot (K_d + w) \cdot d \cdot h \cdot 100}{(K_{d,\mathrm{lit}} + w_{\mathrm{lit}}) \cdot f_s}, \tag{5}$$

where K_d is the adsorption coefficient ($K_d = 50$) [3], and w is the standard soil pore water fraction ($w = 0.15$). The corresponding values from the literature are named $K_{d,\mathrm{lit}}$ and w_{lit}. The density of soils is set at $d = 1.44$ kg/dm^3, and the fraction of herbicide which hits the soil is $f_s = 0.1$. It is assumed that the degradation is similar to natural conditions.

If the test is performed in water solution or in agar the data are converted by the equation:

$$D = \frac{c_{\mathrm{lit}} \cdot (K_d + w) \cdot d \cdot h \cdot 100}{f_s \cdot f_d}. \tag{6}$$

RESULTS

The converted values given in kg/ha are shown in Table 1. The log-normalized values are depicted in Fig. 1. It is evident that the plant data fit a log-normal distribution different from those describing other organism groups. The single value of a nematode is apparently an outlier which may be caused by the test conditions or by extreme insensitivity of this species to glyphosate. This value was removed from the set of data because it did not influence the estimation of a safe environmental concentration value, and it did not belong to the log-normal distributions in question. In the following, plants, animals, and microorganisms were treated as separate groups. The log-normalized values are shown in Fig. 2–4. The plant data show excellent log-normality. The estimated safe concentration $K_p = 0.00025$ kg/ha with a Shapiro-Wilk value $W = 0.98$ (Prob. $< W = 0.97$). The PEC/PNEC ratio for plants is calculated by use of equation (2) using RFD = 1.6 kg a.i./ha resulting in PEC/PNEC = 1.6/0.00025 = 6400.

The microorganisms showed reasonable log-normality with $W = 0.92$ (Prob. $< W = 0.18$), and animals showed poor log-normality with $W = 0.81$ (Prob. $W < 0.07$). The estimated K_p for microorganisms was determined at 61 kg/ha, and for animals at 12 kg/ha, respectively. The corresponding PEC/PNEC ratios were found at 0.026 for microorganisms, and at 0.13 for animals, respectively.

Table 1. Toxicity data on glyphosate showing the species names, NOEC values and units, exposure conditions, toxicity endpoint, exposure time, and NOEC values converted to area based unit D (kg/ha). The references numbers are indicated

Group	Species	NOEC	Unit	Exposure	Endpoint	Exposure time (days)	D-value (Converted NOEC) kg/ha
1	2	3	4	5	6	7	8
Mammals	Dog [3]	> 500	mg/kg/d	oral		365	1760
	Mouse [3]	800	mg/kg/d	oral	growth	730	1250
	Rat [3]	135	mg/kg/d		growth of organs	90	264
Birds	Mallard [3]	> 1000	mg/kg	feed	reproduction		118
	Quail [3]	> 1000	mg/kg	feed	reproduction		118
Earthworms	Earthworm [3]	> 100	mg/kg	in soil	mortality/growth		114
Nematodes	*Heterorhabditis bacteriophora* [17]	> 10000	mg/l	liquid culture	mortality	3	720000
Microorganisms	*Azospirillum lipoferum* [8]	< 5	mg/l	agar	nitrogenase activity	8	361
	Enterobacter agglomerans [8]	5	mg/l	agar	nitrogenase activity	8	361
	Klebsiella pneumonia [8]	5	mg/l	agar	growth	8	361
	Pseudomonas sp. [8]	< 5	mg/l	agar	nitrogenase activity	8	361
	Cenococcum geophilum [6]	10	mg/l	agar	growth	48	722
	Cenococcum graniforme [2]	3590	µg/l	agar	growth	30	259
	Hebeloma crustuliniformes [1]	10	mg/l	liquid culture	growth	30	722
	Hebeloma longicaudum [6]	10	mg/l	agar	growth	48	722
	Laccaria laccata [2]	3590	µg/l	agar	growth	30	259
	Pisolithus tinctorius [6]	< 1	mg/l	agar	growth	26	72
	Suillus tomentosus [2]	8975	µg/l	agar	growth	30	648
	Telephora americana [1]	10	mg/l	liquid culture	growth	30	722
	Telephora terrestris [1]	10	mg/l	liquid culture	growth	30	722
	Cladosporium cladosporioides [24]	2	µg/g	agar	growth rate	27	144
	Paxillus involutus [2]	17.95	mg/l	agar	growth	30	1300
	Phymatotrichum omnivorum [18]	5	µg/ml	agar	growth	7	361

1	2	3	4	5	6	7	8
Plants	*Rhizobium trifolii* [4]	< 2	mg/l	agar	nodulation	56	144
	Vicia faba [20]	< 312	g/ha	spray	growth	42	0.31
	Solanum tuberosum [13]	0.28	kg/ha	spray	growth	14	0.28
	Actinidia chinensis [10]	< 1.2	kg/ha	spray	growth	70	1.2
	Prunus pensylvanica [21]	0.04	kg/ha	spray	mortality	730	0.04
	Saccharum sp. [16]	0.1	kg/ha	spray	growth	28	0.1
	Lycopersicon esculentum [14]	0.004	kg/ha	spray	yield	84	0.004
	Populus tremuloides [21]	< 0.04	kg/ha	spray	mortality	730	0.04
	Zucchini [4]	0.02	kg/ha	spray	phytotoxicity	28	0.02
	Betula papyrifera [22]	< 0.0105	kg/ha	spray	chlorophyll	5	0.0105

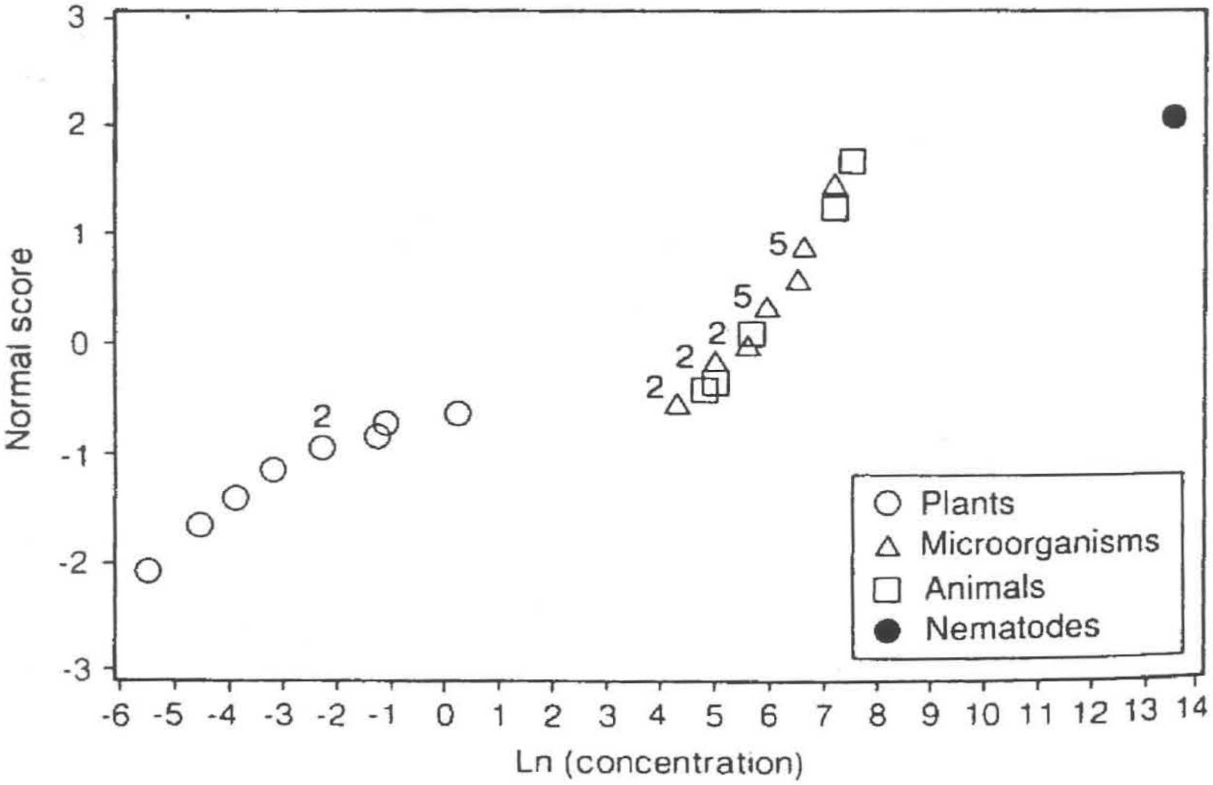

Fig. 1. Log-normal transformed NOEC data on glyphosate given as kg/ha for all organisms. The number of points with identical coordinates are indicated

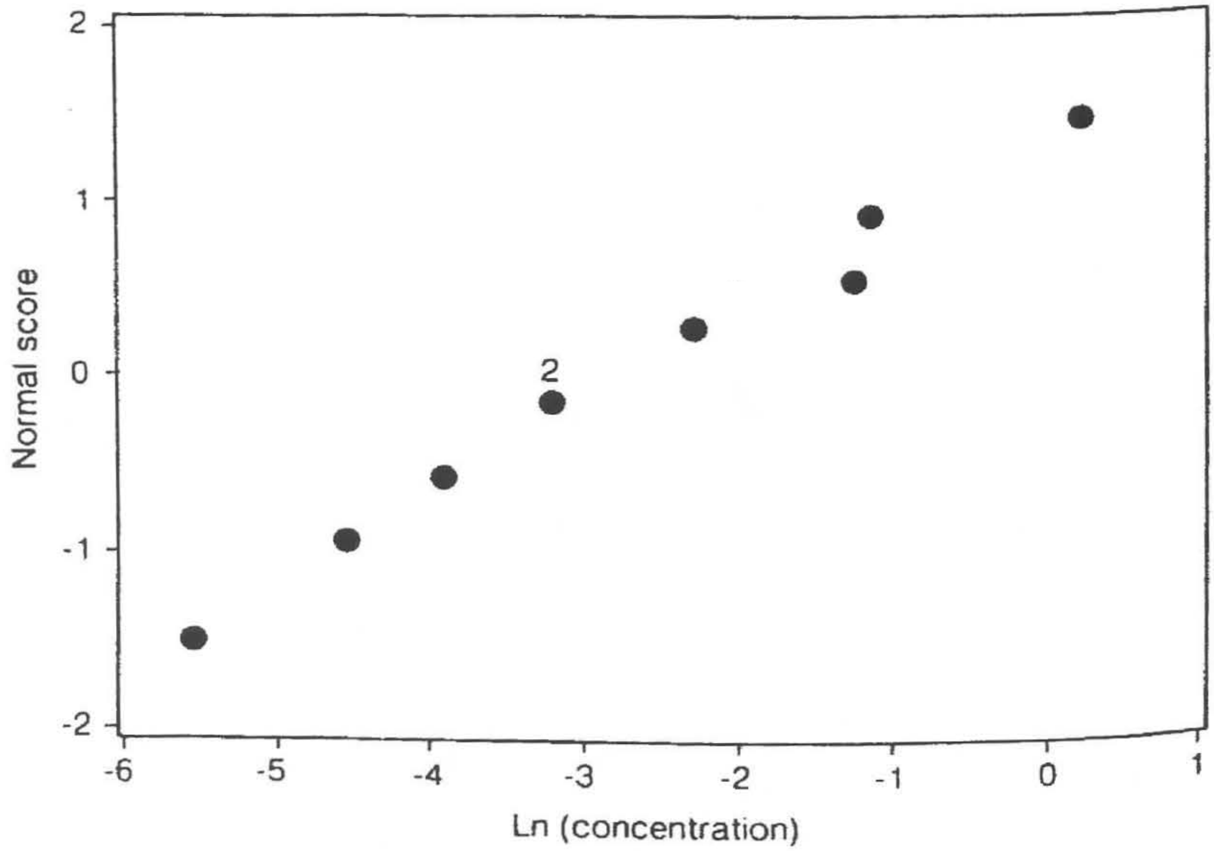

Fig. 2. Log-normal transformed NOEC data for plants given as kg/ha. The number of points with identical coordinates are indicated

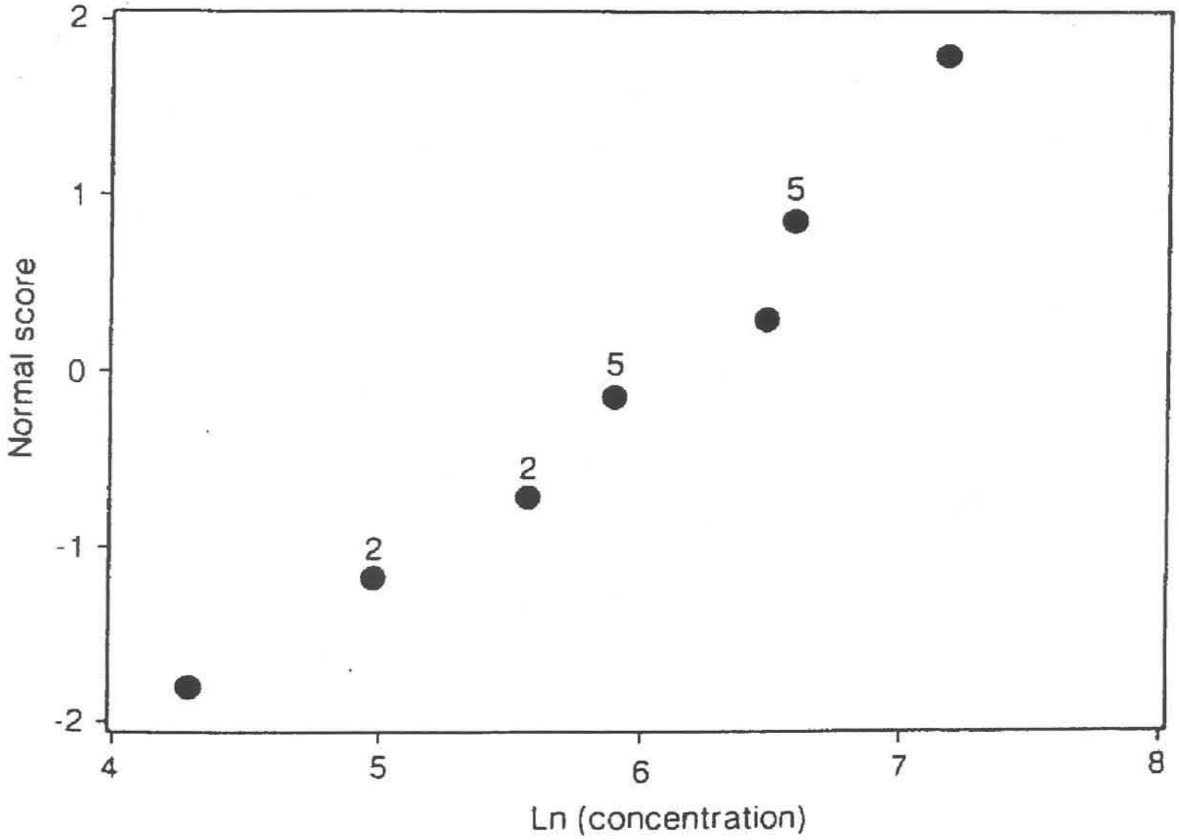

Fig. 3. Log-normal transformed NOEC data for microorganisms given as kg/ha. The number of points with identical coordinates are indicated

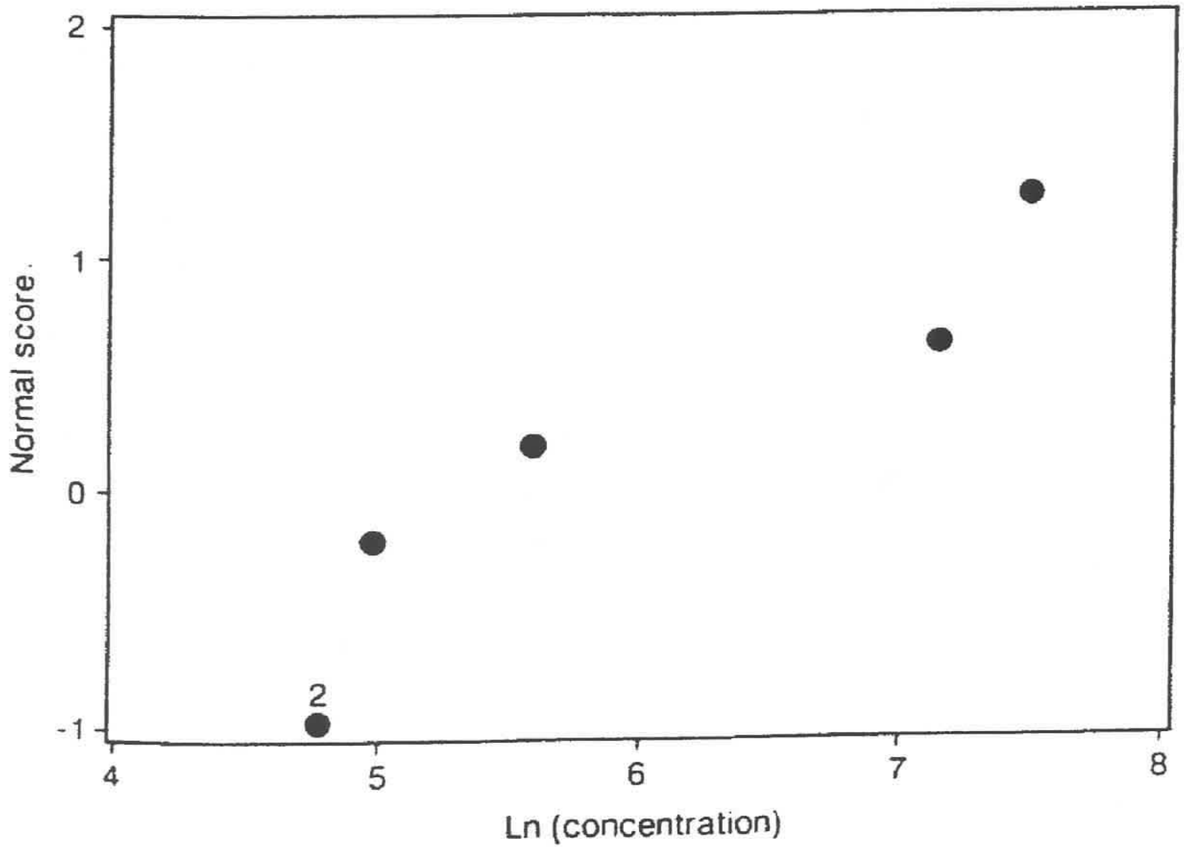

Fig. 4. Log-normal transformed NOEC data for animals given as kg/ha. The number of points with identical coordinates are indicated

DISCUSSION

Recently, there has been much discussion about the theoretical basis of distribution-based extrapolation methods. By use of an extrapolation method, Hopkin found a hazardous concentration for copper which was insufficient to provide for the minimum dietary requirement of the animals in the soil [9]. Some confusion may arise when the bioavailability is not taken into account in the extrapolation process. In most cases, the extrapolation is based on results which are obtained in the laboratory by spiking soil samples with metal salts containing the free active metal cations or other toxic metal ion complexes. This is also the case in the data used by Hopkin which showed extremely low safe concentrations. The active metal species used in the laboratory are not directly comparable with aged deposits under natural conditions which mainly consists of metal oxides and other metal species of very low bioavailability and toxicity.

Forbes and Forbes stated that statistical extrapolation does not presently offer an improvement over much simpler arbitrary assessment factors [7]. They raised questions about the power of the chosen distributions, and about the selection of species and the "representativeness". The present study shows that in the case of glyphosate the single species toxicity test results fit well into log-normal distributions. However, species of largely different taxonomy belong to quite different log-normal distributions. In the present study plants, microorganisms and animals were divided into three groups although the two latter groups might have been combined in one log-normal distribution. The single extreme value of a nematode did not fit into any of the other lognormal distribution used in this study. It can be concluded from the present results that in general it may be useful to consider if data covering large taxonomic distances should be subdivided into smaller groups of organisms before performing hazard or risk assessment by use of distribution-based extrapolation methods.

One of the underlying assumptions of the extrapolation methods is that species selection is random and that the selected species represent the organism group or ecosystem. Forbes and Forbes claim that it may be difficult to design sampling schemes in which random rather than "representative" samples are selected unless the species composition of the community, ecosystem or statistical population of interest has been determined. This is a principal problem. However, the extrapolation method simply tells us that with a given set of data there is a certain probability finding a species which is affected by concentrations lower than the estimated K_p-value. Therefore it is important that the underlying toxicity data cover the whole range of single species toxicities that may occur in an ecosystem or within an organism group. In this context the single species do not represent the ecosystem but rather a range of toxicities.

A proper selection of species still leaves us with the problem that the function of the ecosystem may not be protected by the K_p-value. Further, the interaction between species and with the abiotic environmental conditions may influence the impact of the chemical on the structure and function of an ecosystem. However, in general, the estimated "safe concentrations" are based on conservative assumptions underlying the design of the single species tests and probably, the estimates are over-protective. These aspects need further research, preferably by field validation of the assumptions.

Further, the species should be selected randomly. The taxa should be selected on basis of the knowledge on the species composition of the ecosystem or group of organisms of interest to secure a random selection of species. A priori, by testing a new chemical with unknown toxicity, any single species test can be selected at random among existing tests, each representing a unique and unknown response to that particular chemical. The battery of available tests should be representative in terms of function, taxonomy, trophic level and route of exposure. All these factors require consideration, especially in higher-level testing [5]. Further work is needed to study the question on how to achieve a random selection of species.

From the present study on glyphosate it can be concluded that the plant community is at risk in the field and in areas exposed to spray drift. Apparently, microorganisms and animals are protected from the direct impact of glyphosate at the recommended field dosage level. However, the great impact on the flora component of the ecosystems may indirectly affect the fauna and the microorganisms. Thus the "overall safe concentration" is governed by the assessment of the "safe concentration" for the plant community value at 0.00025 kg/ha, estimated for 95% of the single species with 95% probability.

Acknowledgements: The National Pesticide Research Fund at the Danish Environmental Protection Agency is thanked for partly financial support of the project.

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EXTRAPOLATION OF THE EFFECTS OF GLYPHOSATE FROM THE LABORATORY TO THE FIELD

Summary

A method is presented for the extrapolation of single species toxicity data on pesticides from the laboratory to the field. The extrapolation was performed on data from the literature on the herbicide glyphosate which were converted to an area-based unit. The statistical extrapolation method used is based on the assumption that the data follow a log-normal distribution. The statistical analysis showed that to obtain log-normality, the data should be treated in three separate groups of organisms; plants, microorganisms, and animals, respectively. 'Safe concentrations' for the environment was estimated as the exposure/ecotoxicity ratio, PEC/PNEC. The recommended dosage of glyphosate is 1.6 kg/ha. The PEC/PNEC ratios found for microorganisms and animals were 0.026 and 0.13, respectively. Thus, these groups were found to be protected from the direct effects of glyphosate. The overall safe concentration was governed by the "safe concentration" for 95% of the plant species which was estimated with 95% probability at the value 0.25 g/ha corresponding to a PEC/PNEC ratio of 6400.

EKSTRAPOLACJA EFEKTÓW GLIFOSATU Z LABORATORIUM W TEREN

Streszczenie

Przedstawiono metodę ekstrapolacji danych toksyczności pojedynczego gatunku dotyczących pestycydów z laboratorium w teren. Ekstrapolacji dokonano na danych z literatury dotyczących glifosatu herbicydowego, które przekształcono w jednostkę powierzchniową. Zastosowana metoda ekstrapolacji statystycznej opiera się na założeniu, że dane podlegają normalnemu rozdziałowi logarytmicznemu. Analiza statystyczna wykazała, że aby uzyskać normalność logarytmiczną, dane należy traktować w trzech osobnych grupach organizmów: rośliny, mikroorganizmy i zwierzęta. „Bezpieczne stężenia" dla środowiska oszacowano jako stosunek ekspozycja/ekotoksyczność, PEC/PNEC.

Zalecana dawka glifosatu wynosi 1,6 kg/ha. Stosunki PEC/PNEC stwierdzone dla mikroorganizmów i zwierząt wyniosły odpowiednio 0,026 i 0,13. Stąd stwierdzono, że grupy te są chronione przed bezpośrednim wpływem glifosatu. Całkowite bezpieczne stężenie kontrolowano przez „bezpieczne stężenie" dla 95% gatunków roślin i oszacowano z 95% prawdopodobieństwem na 0,25 g/ha, co odpowiada stosunkowi PEC/PNEC 6400.

ЭКСТРАПОЛЯЦИЯ ЭФФЕКТОВ ГЛИФОСАТА ИЗ ЛАБОРАТОРИИ В ПОЛЕ

Резюме

Представили метод экстраполяции данных токсичности отдельного вида, касающихся пестицидов из лаборатории в поле. Экстраполяцию проводили по литературным данным, относящимся к гербицидному глифосату, которые превратили в единицу поверхности. Применяемый метод статистической экстраполяци основывается на предположению, что данные подчиняются логнормальному распределению. Статистический анализ показал, что для получения логарифмической нормальности следует данные считать в трех отдельных группах организмов: растения, микроорганизмы и животные. „Безопасные концентрации" для среды определили как отношение экспозиция/экотоксичность, PEC/PNEC.

Рекомендуемая доза глифосата составляет 1,6 кг/га. Соотношения PEC/PNEC, отмеченные для микроорганизмов и животных составляли соответственно 0,026 и 0,13. Отсюда делается вывод, что эти группы охраняются от непосредственного влияния глифосата. Валовая безопасная концентрация контролировалась „безопасной концентрацией" для 95% видов растений, что было определено с вероятностью 95% на 0,25 г/га, что соответствует отношению PEC/PNEC 6400.